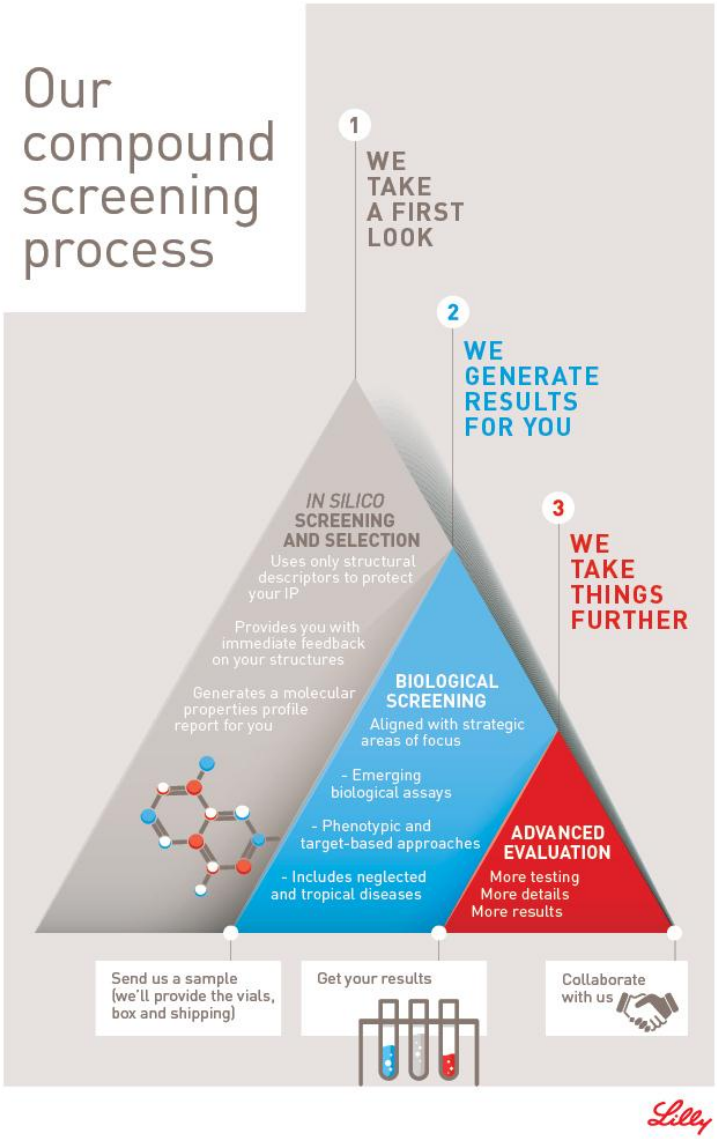
Understanding Your Data:

A User Guide to Interpreting Data Provided by the Lilly OIDD Program

Overview

The goal of this document is to help investigators at OIDD-affiliated institutions to understand the biological data produced in *in vitro* screening. Member investigators whose compounds pass *in silico* screening will be invited to submit samples for the more robust biological screening process and will then receive a final biological results report.



The OIDD screening panel comprises a series of assay modules which are relevant to therapeutic areas of long-term strategic interest to Lilly. The screening panel includes both phenotypic as well as target-based modules.

Each screening module consists of a series of primary, secondary and confirmatory assays and is designed to define the compound's activity profile and early potential for further optimization. The results from the primary assay in each screening module will determine whether the compound is run through the secondary assay screens and beyond.

Any compound submitted and accepted will be evaluated in at least all of the primary assay screens, and data generated will be returned to the investigator in the form of a personalized data report.

Primary Screening Assays: Single Point mode

The first data generated is a primary Single Point assay or SP. This assay is performed with only one concentration of the test substance. The purpose of the assay is to find out if compounds are active, and thus triage quickly those that are not. We typically test the compound in the designed assay (varies with each module) and measure the response based on a biomarker, inhibition, stimulation, blockade, agonist/antagonist, etc.

The SP assay is reported as percent vs maximal response (inhibition/stimulation) at a given concentration. The higher the value is, the higher the activity of the compound (Equations 1 and 2).

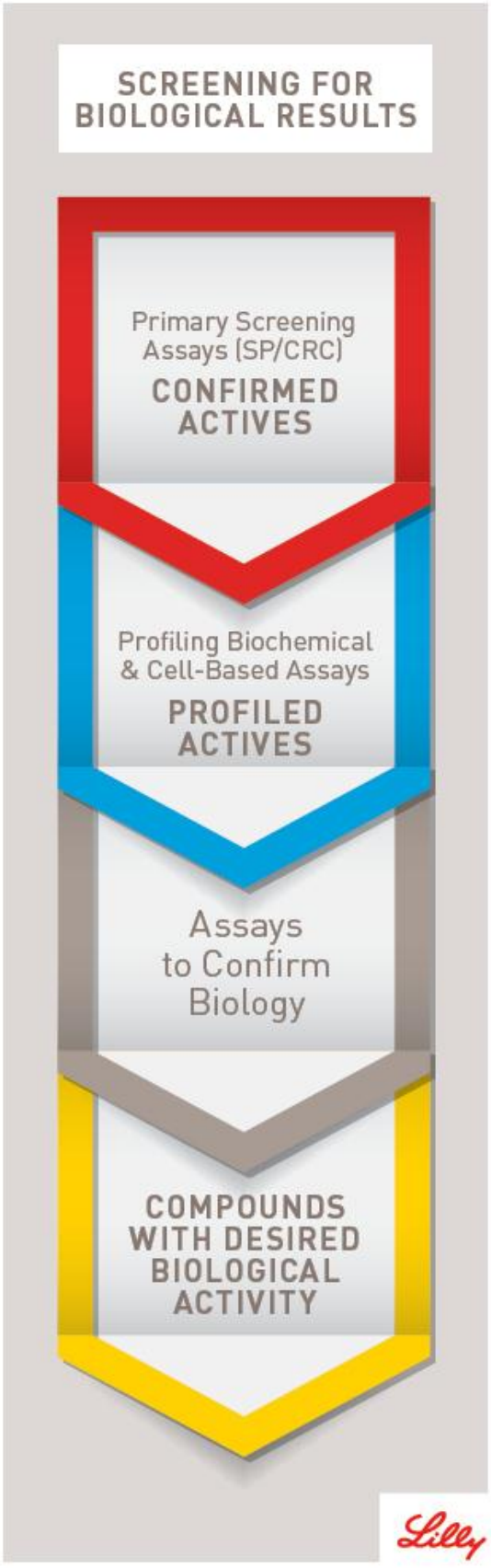
Each assay will have a cut off value specified; thus, only compounds above that value will pass to next filter. A typical cut off value is 50%, but this may vary depending on the assay.

Negative values are interpreted, in most cases, as lack of compound activity. This is because stimulation/inhibition is calculated based on the compound's response in comparison to the maximal and minimal signal in the assay (see Equations 1 & 2).

Hit rate of compounds in SP largely varies depending on the assay, but it is typically between 0.5 and 5%. It is also worthy to note that there is intrinsic variability in these assays that may lead to false positive results. In addition, it is possible that these false positives are due to artefacts, such as interference with detection technology, compound aggregation, compound purity, etc. Therefore, it is important to confirm activity of the compound in concentration response mode, as well as in other assays in the flowscheme.

$$\textit{Equation 1: Stimulation (\%)} = \frac{Signal - Min}{Max - Min} \times 100$$

$$\textit{Equation 2: Inhibition (\%)} = \left[1 - \left(\frac{Signal - Min}{Max - Min}\right)\right] \times 100$$



Primary Screening Assays: Concentration-Response Curves

Those compounds that show SP values within established specifications will be tested in a Concentration Response Curve or CRC mode. The purpose of this second test in the primary assay is to confirm compound activity from SP and select the compounds that should progress to the secondary screening (Figure 2). To generate a CRC, increasing concentrations of the compound are tested and their effects measured. Then, the compound concentration that produces half-maximal response in the assay is determined. This value is referred as EC50 for activation and IC50 for inhibition assays, which are considered a measure of compound potency. The lower the AC50 value (IC50 or EC50) the less compound is necessary to elucidate a response and the higher the potency of the compound. When the compound is active, we observe a graph such as in Figure 3, where the % of inhibition or stimulation increases with the concentration until the maximal response is achieved.

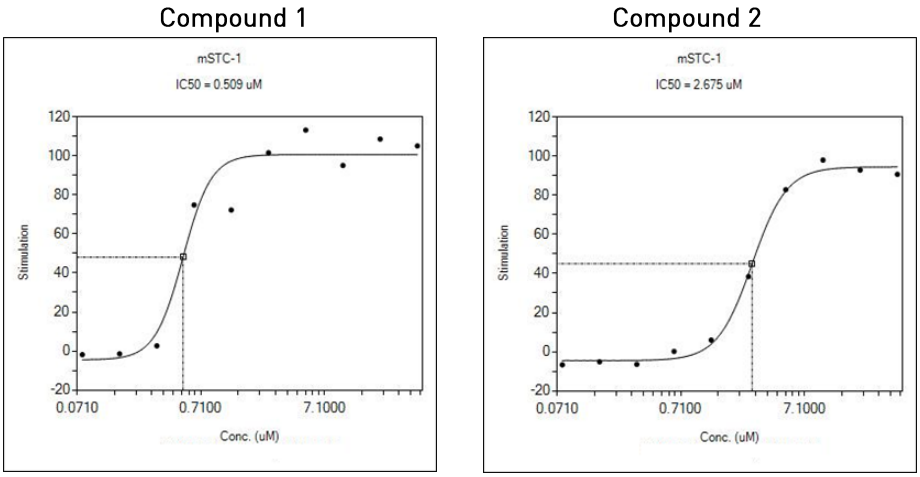


Figure 3 – These are two examples of "good curves," the type of curves that you should expect to see if the compounds are active. *Note that compound 1 is more potent than compound 2.*

Functional responses: Agonist vs Antagonist

A compound is considered an agonist when it binds to a receptor and activates it to produce a biological response that may (or may not) be the same as the biological response caused by its natural ligand.

A full agonist is a compound that produces the system maximal response, whereas a partial agonist produces a lower maximal response. By definition, a partial agonist has lower intrinsic efficacy (power of the agonist to produce response) than a full agonist, even at compound concentrations where all receptors are occupied. That is shown in the maximal response (top asymptote) elicited by each compound. Figure 4b shows a full agonist (blue), a partial agonist (green) and an inactive compound (red).

A compound is an antagonist when it blocks or impedes agonist-mediated responses and thus eliminates or reduces the activity of the target enzyme or receptor. Antagonist activity is measured in the presence of an agonist (typically the endogenous ligand). The activity can be expressed as % inhibition and both IC50 value and % maximal inhibition are calculated.

Potency vs. Efficacy

Concentration response curves may provide information on both potency and efficacy. It is important to differentiate between both concepts.

Potency refers to the compound concentration required to produce an effect, whereas efficacy is the ability of the compound to provoke a change when it binds to the target.

As previously discussed, by measuring the maximal response (top asymptote) of two agonists, in the same system, it is possible to calculate their relative efficacy (efficacy of test compound vs efficacy of internal standard). The higher the maximal response, the higher the efficacy of the compound tested. However, once the maximal possible response in a biological system is achieved, it is not possible to distinguish differences in efficacy.

Figure 4 illustrates these concepts; in Figure 4a, the blue curve represents a more potent compound than the green curve, but since both compounds have elicited the maximal possible response in the system, their relative levels of efficacy cannot be differentiated. Both compounds are full agonists in this system, but they may have different intrinsic efficacies. In Figure 4b, the blue curve represents a compound with higher efficacy than the compound represented by the green curve (partial agonist), but both show similar potency. The red curve represents an inactive compound

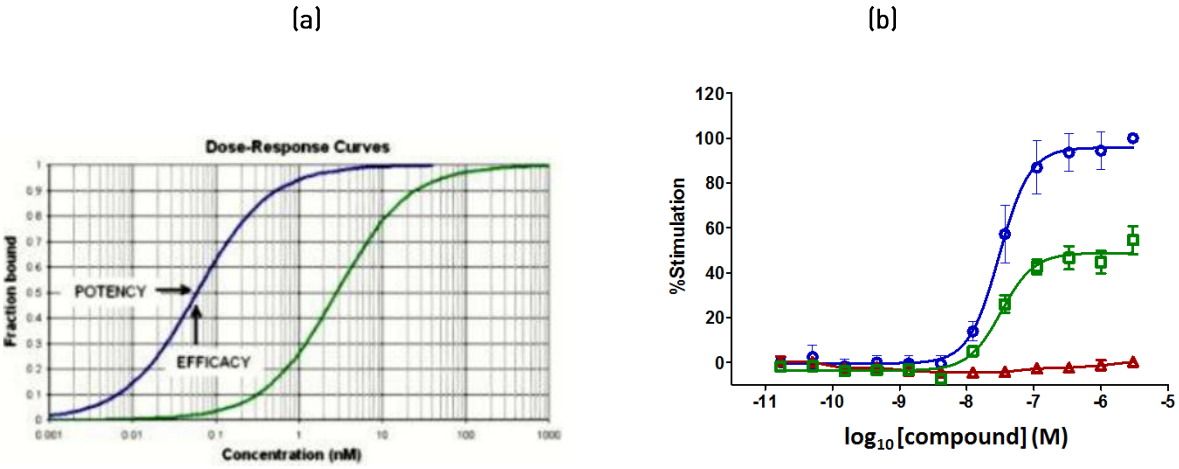


Figure 4 – (a) Concentration-response curves (or CRC) of two theoretical compounds. (b) CRC of a real example.

On the other hand, when we have curves such as the ones represented in **Figure 5b and 5c**, we can interpret that the compound tested shows poor potency, as very high concentrations are required to provoke a response.

(a) (b) (c)

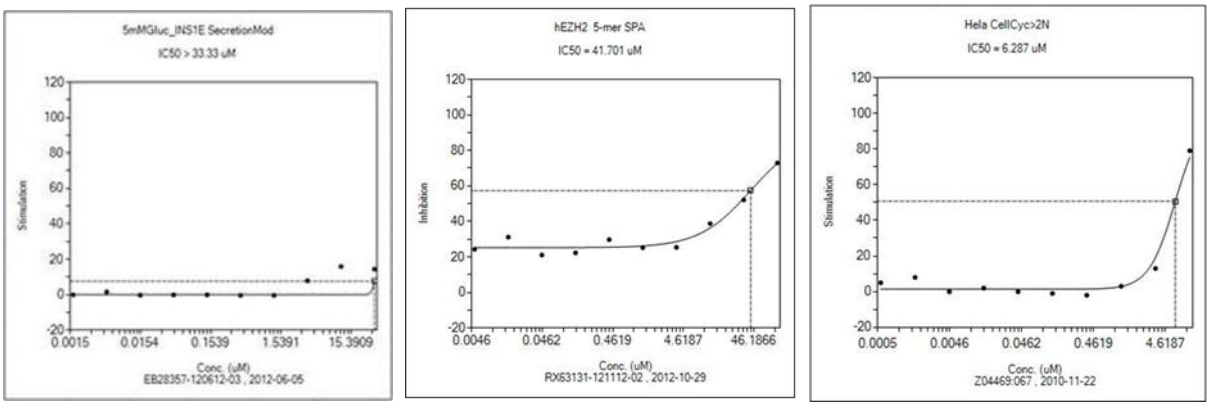


Figure 5 – **(a)** Curve would be ideal result for a counter screen or "off-target" assay because lack of activity would be the desired result. **(b)** and **(c)** A high concentration of substrate is required to elucidate a response.

Using the data for publication

We encourage you to use the data provided by the OIDD program to further your research and to publish. Please contact the OIDD team (openinnovation@lilly.com) to share your plans, and we will provide additional information on methods, material, assays, data interpretation, or other needs.